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Absence of a relationship between endometriosis and the N314D polymorphism of galactose-1-phosphate uridyl transferase in a UK population

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An association between the N314D polymorphism of galactose-1-phosphate uridyl transferase and endometriosis has recently been reported in a North American population. To determine whether such an association exists in the UK population, we genotyped 148 women with sporadic (*n* = 91) or familial (*n* = 57) endometriosis, a control population of 95 male blood donors and a control group of 53 women with a normal pelvis at hysterectomy. Heterozygosity for the polymorphism was found in 14.9% (22/148) of affected women, 13.7% (13/95) of male blood donors and 11.3% (6/53) of women with a normal pelvis. There was no statistically significant difference in the frequency of the polymorphism between cases and controls in the UK population, even when the cases were divided into groups of moderate–severe disease, sporadic cases or familial cases. We conclude that the galactose-1-phosphate uridyl transferase N314D polymorphism is unlikely to be associated with endometriosis in the UK population.

Key words: endometriosis/galactose-1-phosphate uridyl transferase/genetics

Introduction

It is likely that endometriosis has a genetic basis (Kennedy, 1997) as it has a familial tendency (Kennedy *et al*., 1995) and first-degree relatives of affected women have an increased risk of developing the disease (Simpson *et al*., 1980; Moen and Magnus, 1993). The prevalence of endometriosis may be as high as 15% in the sisters of women with moderate to severe disease (Kennedy *et al*., 1998). Recently, three studies have shown associations between endometriosis and polymorphisms in candidate genes (Baranov *et al*., 1996, 1997; Cramer *et al*., 1996b). Cramer *et al*. (1996b) reported an association between the N314D polymorphism of the galactose-1-phosphate uridyl transferase (GALT) enzyme in a North American population. However, Morland *et al*. (1998) have failed to reproduce this finding in a sample drawn from the UK population.

The N314D polymorphism results from an A to G transition in exon 10 of the gene, substituting aspartate for asparagine. GALT is involved in the breakdown of galactose and the N314D polymorphism generally causes reduced enzyme activity. The polymorphism has previously been associated with infertility, premature ovarian failure, Müllerian anomalies (Cramer *et al*., 1989, 1996a) and ovarian cancer (Morland *et al*., 1998).

Reduced GALT activity associated with N314D may be an aetiological factor in endometriosis or the polymorphism may be in linkage disequilibrium with a disease susceptibility allele. The *GALT* gene locus has been mapped to the short arm of chromosome 9p21 (Bricarelli *et al*., 1981), a region where loss of heterozygosity at candidate ovarian tumour suppressor loci has been reported in endometriotic tissue (Jiang *et al*., 1996). Another possibility is that maternal GALT deficiency could increase the risk of endometriosis in female offspring. This is supported by the fact that the female offspring of rats fed a high galactose diet had delayed vaginal opening and reduced oocyte number (Chen *et al*., 1981) and that case reports have shown a relationship between Müllerian aplasia and maternal GALT deficiency (Cramer *et al*., 1987).

We aimed to determine whether the polymorphism is associated with endometriosis in a UK population. Women with endometriosis and mothers of women with endometriosis were genotyped and compared to both a population frequency and a control group of women without endometriosis at hysterectomy. As the population frequency for the N314D polymorphism is unknown in the UK (L.Tyfield, personal communication) we estimated the prevalence by genotyping male blood donors.

Materials and methods

Blood or tissue was obtained from the following five groups and DNA extracted for genotyping: (A) male blood donors (*n* = 95), (B) pre-menopausal women aged 40–50 years with a normal pelvis at hysterectomy (*n* = 53), (C) sporadic cases with histologically confirmed endometriosis (*n* = 91), (D) women with moderate–severe (stage III–IV) (AFS, American Fertility Society, 1995) endometriosis and a family history of the disease (*n* = 57), but unrelated to each other, and (E) mothers of women with moderate–severe endometriosis (*n* = 30). The Central Oxford Research Ethics Committee approved the collection of blood samples from familial cases and their parents.

(A) Population controls

Samples from males (*n* = 95) who had donated blood in the Oxford region were supplied anonymously by the National Blood Service.

(B) Normal controls

Pre-menopausal women ($n = 53$) aged between 40 and 50 years with a normal pelvis at hysterectomy were identified through the records of the John Radcliffe Hospital, Oxford. Women from this age group were chosen to maximize the probability that they were unaffected by endometriosis, instead of younger women who might develop the disease in later life. Normal tissue, fixed in formalin and paraffin-embedded, from these patients was provided anonymously from the archives of the Cellular Pathology Department at the John Radcliffe Hospital.

(C) Sporadic cases

Women with histologically confirmed endometriosis ($n = 91$) were identified from histopathology reports and samples of normal, formalin-fixed, paraffin-embedded tissue were provided anonymously as for group B.

(D) Familial cases

Families with endometriosis, including sisters, mother–daughter pairs and other pedigrees are being recruited for the Oxford Endometriosis Gene study (OXEGENE) (Kennedy, 1997). The purpose of OXEGENE is to identify susceptibility loci for endometriosis by genetic linkage analysis; blood samples have therefore been obtained and DNA isolated from members of the affected pedigrees recruited. DNA from only one individual, recruited in the UK, with stage III–IV disease from each family was studied ($n = 57$). The mean current age of the women studied was 43.9 years (range 27–64).

(E) Mothers of affected women

Blood samples were obtained from the mothers of women with stage III–IV endometriosis in group D ($n = 30$). Two of the mothers (7%) also had endometriosis.

Genotyping

Genomic DNA was extracted from 9 ml of EDTA anti-coagulated whole blood using phenol–chloroform (patient groups D and E) or using the QIAmp DNA extraction kit (Qiagen) (group A). For groups B and C, DNA was extracted from paraffin-embedded, formalin-fixed, tissue samples using the QIAmp tissue kit (Qiagen, Crawley, UK). The N314D genotype of the individual was determined using a previously published method (Lin *et al*., 1994). Briefly, a 311 bp fragment of exon 10 of the *GALT* gene was amplified by polymerase chain reaction (PCR) and then subjected to restriction enzyme analysis with *Ava*II (Boehringer Mannheim) with visualization on a NuSieve® 3:1 agarose gel (FMC, Rockland, ME, USA). The region of exon 10 amplified contains one *Ava*II restriction site normally, resulting in bands of 215 and 96 bp. This provides an internal control of enzyme digestion and two restriction sites if the polymorphism is present, resulting in bands of 215, 111, 104 and 96 bp as shown in Figure 1.

Statistical comparisons were made using Fisher's exact test.

Results

No individual tested was found to be homozygous for the N314D polymorphism. The estimated population frequency of heterozygosity for the polymorphism in the UK, determined by testing 95 male blood donors, was 13/95 [13.7%, 95% confidence interval (CI) 7.5–22.3%].

For all cases with endometriosis (groups C and D), 22/148 (14.9%, 95% CI 9.6–21.6%) were heterozygous for the N314D polymorphism. In comparison, 6/53 (11.3%, 95% CI 4.3–23.1%) women with a normal pelvis at hysterectomy (group B) were heterozygous for the polymorphism. The frequency in cases was not significantly different to the frequency in either the male blood donors or in women with a normal pelvis at hysterectomy ($P = 0.85$ and 0.65 respectively). Nor was there a significant difference in the frequency amongst the mothers of women with rAFS stage III–IV endometriosis (group E) compared to the control groups as 5/30 (17%) were identified as being heterozygous for the polymorphism ($P = 0.76$ and 0.52 respectively). Both mothers who also had endometriosis lacked the N314D polymorphism.

The cases were divided into groups of stage III–IV disease only and sporadic or familial cases only, but no statistically significant differences were observed in the frequency of the polymorphism between any of these groups and the controls (Table I).

Among the familial cases (group D), 28 women reported that they were infertile of whom eight (29%) carried the N314D polymorphism. Of the 18 women in group D who reported no problems conceiving, only one (6%) carried the polymorphism. Eleven of the familial cases did not know or did not report their fertility status. Data on fertility status were not available for the sporadic cases.

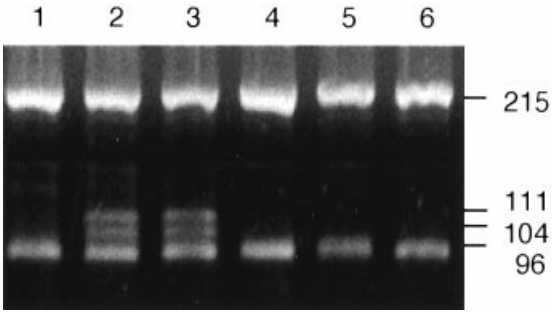

Figure 1. Ethidium bromide stained NuSieve® 3:1 agarose 4% gel electrophoresis of the products of *Ava*II digestion following polymerase chain reaction amplification of a 311 bp fragment of exon 10 of the *GALT* gene. Normally there is one restriction site (resulting in bands of 215 and 96 bp) but the A to G transition results in a new restriction site. Lanes 2 and 3 show individuals heterozygous for the polymorphism, with bands of 215, 111, 104 and 96 bp.

Table I. Prevalence of the N314D polymorphism of galactose-1-phosphate uridyl transferase in women with endometriosis and controls

Subjects	*n*	Heterozygous for N314D polymorphism (%)	95% CI
Controls			
Male controls	95	13 (13.7)	7.5–22.3
Women with a normal pelvis at hysterectomy	53	6 (11.3)	4.3–23.1
Cases			
All cases[a]	148	22 (14.9)	9.6–21.6
Moderate–severe disease only	102	17 (16.7)	10.0–5.4
Sporadic cases only	91	13 (14.3)	7.8–23.2
Familial cases only	57	9 (15.8)	7.5–27.9

[a]All cases defined as sporadic and familial cases.
CI = confidence interval.

Discussion

We have not found an association between the *GALT* N314D polymorphism and endometriosis in this UK population, nor have we found that mothers of women with disease were more likely to carry the polymorphism than the general population.

Our findings contrast with those of Cramer *et al*. (1996b) who reported that 10/33 (30%) of North American women with all stages of endometriosis carried the N314D polymorphism compared to 15/111 (14%) pre-menopausal, female controls. Moreover, in those cases with stage III–IV disease, 6/11 (54%) carried the N314D polymorphism. There are several possible reasons for the observed differences between this study and our findings. Firstly, these may represent actual genetic differences between the North American and UK populations. Secondly, the sample size in the study by Cramer *et al*. was small and the findings may represent a type I error. Lastly, the cases studied were attending a fertility clinic and the polymorphism is already known to be associated with infertility (Cramer *et al*., 1989) which may have introduced bias. Our finding that 8/28 (29%) women with endometriosis who reported infertility were heterozygous for the N314D polymorphism, in combination with Cramer's findings, suggests that the relationship between GALT N314D, infertility and endometriosis requires further investigation.

Our findings are consistent with those of Morland *et al*. (1998) who reported that 14/78 (18%) endometriosis cases carried the N314D polymorphism compared to 42/248 (17%) controls in the UK. The frequency of the polymorphism in these controls was not significantly different to our control group of blood donors ($P = 0.51$) or women with a normal pelvis at hysterectomy ($P = 0.41$).

There can be a number of reasons why a disease and a marker allele are associated. Firstly, the marker allele may directly result in an increased susceptibility for the disease. Secondly, although over many generations multiple, random, recombination events occur along a chromosome they are less likely to occur between alleles within $<$200 kb of each other. Therefore, the marker allele and disease, if very close to one another, may be in linkage disequilibrium. Thirdly, it is possible that the affected individuals are a genetically unique subset of the population. In the investigation of cancer and other chronic diseases such as multiple sclerosis, the reproducibility of candidate loci association studies has been poor (Ebers and Dessa Sadovnick, 1994). The ability to replicate candidate gene studies, to confirm associations, is particularly important in defining disease aetiology. The lessons learned from experience in other diseases should be taken into account in this new area of research into the causes of endometriosis.

Nevertheless, the considerable evidence that endometriosis has a genetic basis (Simpson *et al*., 1980; Moen and Magnus, 1993; Kennedy, 1997) warrants the further investigation of other candidate genes. There is evidence for example of a relationship between endometriosis and a null mutation in glutathione-S-transferase mu 1 (*GSTM1*), caused by a 10 kb deletion in the 1p13 region (Baranov *et al*., 1996; Baranova *et al*., 1997). Baranova *et al*. (1997) found that 86% (43/50) of affected (rAFS stages I–IV) women were homozygous for the null mutation compared to 46% (33/72) of controls. This association may be causative, and other enzymes under the control of the aryl hydrocarbon receptor, which are upregulated by dioxin and other chemical compounds, should also be considered.

The region of chromosome 9p21 to which the *GALT* gene maps (Bricarelli *et al*., 1981) is a region where loss of heterozygosity at candidate ovarian tumour suppressor loci has been reported in endometriotic tissue (Jiang *et al*., 1996). Loss of heterozygosity on chromosome 9p was present in the endometriotic tissue of 8/40 (18%) cases. Loss of heterozygosity on chromosome arms 11q and 22q was also detected, suggesting that these areas may also be worthy of further investigation. There are a number of other GALT polymorphisms, for example Q188R, which has a very low population frequency but accounts for 60–70% of all cases of classical galactosaemia.

In conclusion, we have not found an association between the *GALT* N314D polymorphism and endometriosis in the UK population, although our study may have some limitations. The sample sizes may be too small to detect very small differences between the proportions of cases and controls with the N314D polymorphism.

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